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Flexible Scaffolding Made of Rigid BARs

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Crescent-shaped BAR domains are generic actors in the creation of membrane curvature. In this issue, Frost et al. (2008) reveal how collective twisting of rigid F-BAR domains on a soft membrane surface may lead to different membrane curvatures.

It is easy to imagine how constraining a cellular membrane within a rigid protein scaffold controls membrane geometry (Zimmerberg and Kozlov, 2006; McMahon and Gallop, 2005). Given this, the molecular design of domains of the BAR protein superfamily immediately suggests that their function is to form scaffolds that enable membranes to curve. "Classical" N-BAR and F-BAR domains are elongated curved dimers that strongly bind to membranes through several patches of basic residues distributed on their concave surfaces (Figure 1A). The membrane affinity of the arc of the N-BAR domain is further enhanced by amphipathic wedges (Gallop et al., 2006). Hence BAR domains are believed to curve membranes along their interaction faces (Figure 1A). Cellular expression of different BAR proteins or massive adhesion of BAR domains to pure lipid membranes cause membrane tubulation, with tubule radii increasing with decreasing curvature of the BAR domains as determined in protein crystals (Zimmerberg and McLaughlin, 2004; Itoh and De Camilli, 2006; Masuda et al., 2006). This scaling suggests that the BAR domain might be a molecular scaffold that bends membrane in accordance with its shape.

A single crescent, however, does not a tube make as Frost et al. (2008) now report in this issue. A high surface density of BAR domains is required to initiate membrane tabulation, suggesting cooperativity between individual BAR domains, either through direct protein-protein interactions, membrane-mediated interactions, or spatial ordering of the domains driven by protein crowding (Reynwar et al., 2007; Shnyrova et al., 2007). All of these interactions can contribute to membrane bending. Moreover, shallow insertion of an amphipathic helix as occurs with the N-BAR domain (Gallop et al., 2006) induces local bending stresses that generally do not depend on protein shape but rather rely on perturbation of the target lipid monolayer into which the amphipathic helix is inserted (Zimmerberg and Kozlov, 2006). Thus, the mechanism of curvature creation during membrane tubulation might advance far beyond formation of a molecular scaffold by seemingly curved molecules. And what stabilizes the wrapping of these partial ring elements around tubes of variable sizes?

Using (cryo)electron microscopy, Frost et al. (2008) set out to address these questions. They characterize an F-BAR coated tube crystallized on lipid templates of different rigidity and present a set of distinct molecular interactions revealed by solving the membrane pattern structure of two BAR domains, FBP17 and CIP4, in these 2D crystals. They report that F-BAR can be differentially oriented on a membrane surface either to lie flat on its flat side on a flat membrane or to lie curved on its concave side on a curved membrane. This was unexpected because usually it is thought that the concave surface is the only one designed for mem-

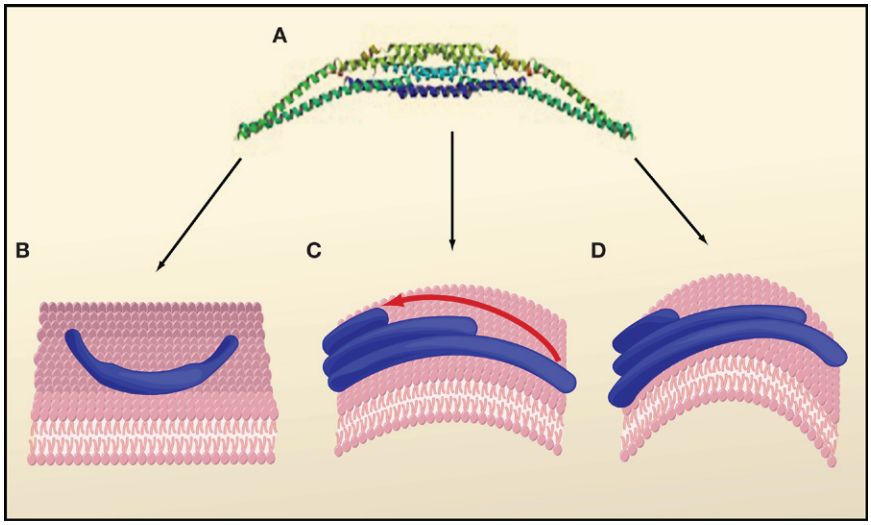

Figure 1. F-BAR Twisting Coupled to Membrane Curvature
(A) Ribbon representation of F-BAR domain architecture.
(B) Binding of an F-BAR dimer on its side to rigid "unbendable" membrane (Frost et al., 2008).
(C) Cooperative flipping of F-BAR dimers into an upright position creates a cylindrical shape; the dimers interact through opposite domains so that chains of the dimers are wound along the helical line (red arrow), thus imposing a cylindrical but not a hemicylindrical shape.
(D) Twisting of the F-BAR domain on the narrow cylinder: the F-BAR domain is not oriented along the arc of maximum curvature as in (C), but the curvature of the attachment line between F-BAR and the cylinder is preserved, and so is the shape of the F-BAR (Frost et al., 2008).

brane binding, and that deformation of the protein would be needed when it binds to a rigid flat membrane. Interaction of the F-BAR modules of FBP17 with rigid membranes frozen in the gel state at 4°C results in flat membrane surfaces covered with F-BAR domains bound through a side interface (Figure 1B) rather than their concave surface. Although there is no large basic patch on the side surface, mutation analysis identified several positively charged residues (K56, R104, and others) that are critical for binding through this interface, but these are different from the residues that are essential for membrane tubulation (Frost et al., 2008).

On soft templates, self-assembly of F-BAR modules of CIP4 causes membrane tubulation. The tubulation efficiency depends mainly on basic patches facing the membrane (Shimada et al., 2007; Frost et al., 2008), although F-BAR domains may also show insertion of some residues at points of membrane and protein contact that are too shallow to be seen by (cryo)electron microscopy. Shallow insertions greatly stimulate tubulation as has been shown for proteins with N-BAR domains (Gallop et al., 2006). Slow temperature annealing of F-BAR tubules results in cylindrical crystals where the concave interface of F-BAR domains faces the membrane (Figure 1C). In these crystals, Frost et al. (2008) discover extensive interaction networks between F-BAR domains. Although some of these interactions can be direct consequences of the annealing process, the authors found that membrane tubulation critically depends on highly conserved residues mediating lateral (e.g., K66-E285 and K273-D286 pairs, F276, and others) and tip-to-tip (K166) interactions between the BAR domains in a crystal. These interactions spread along two distinct helical patterns: lateral attraction causes dimer stacking along its left-handed helical path whereas tip-to-tip interaction spreads along the thread with a shallow right-handed twist so that the dimer arc is oriented almost along the line of maximum membrane curvature (Figure 1C). This orientation corroborates the agreement between the tubule radius and the curvature of the F-BAR domain.

Despite these extensive protein-protein interactions, however, the F-BAR lattice displays flexibility manifested in a relatively wide distribution of tubule sizes (Frost et al., 2008). Such flexibility, likely even more pronounced without annealing, is more consistent with a set of weak nonspecific interactions between proteins bound to the membrane at high surface density rather than specific and rigid lock-and-key type arrangements. Indeed, Frost et al. (2008) reveal that tip-to-tip attraction reportedly supporting formation of long F-BAR filaments (Shimada et al., 2007) is rather labile and has to be omitted to fit the F-BAR distribution on narrow tubules. Their analysis indicates that tubule narrowing correlates with left-handed tilting of F-BAR dimers relative to the axes of the tubule (Figure 1D), as suggested earlier (Henne et al., 2007). Frost et al. (2008) note that this rotation breaks tip-to-tip contacts but surprisingly preserves key lateral interactions. Changing the F-BAR domain orientation on narrow tubules is necessary to preserve the geometry of F-BAR contact with the membrane as in the tilted state the curvature of the membrane-binding concave face of the protein is preserved (Figure 1D). However, tilting of the F-BAR dimers is unlikely to explain formation of the narrow 20 nm tubes reported earlier (Henne et al., 2007).

The findings by Frost et al. (2008) ascribe substantial power to the lateral interactions between F-BAR domains. The authors suggest that the lateral attraction, at sufficiently high surface density of F-BAR, causes cooperative flipping of F-BAR domains lying on their side to an upright position (Figure 1C) so that they impose membrane curvature. The curvature is stabilized by arranging a helical stack of F-BAR dimers by lateral adhesion, and cooperative twisting of the dimers in the stack further regulates stack geometry (Figure 1D). Thus the curvature scaffold is actually a whole polymer (Shimada et al., 2007), not a single polymer unit. The rigidity of the polymerized scaffold relies greatly on long-range interactions between polymer units, not on the shape of the units. That might explain why N-BAR domains can create tubules with radii generally larger than predicted from molecular curvature (Gallop et al., 2006). We note that the term rigidity should be used with caution when applied to curvature scaffolding as the rigidity can vary for two principal curvatures of the surface. Estimates of the rigidity of tubular scaffolds by their persistent length (Frost et al., 2008) are instructive but are not a direct measure of the strength of the set of intermolecular bonds involved in curvature creation.

If high cooperativity between F-BAR dimers is indeed required for membrane bending than individual F-BARs would prefer an orientation lying on their side thus causing no curvature (although the sideways binding has yet to be confirmed for F-BAR modules other than FBP17). Then curvature creation by F-BAR domains will strongly depend on their protein partners, membrane rigidity, and, probably, dynamic features of proteolipid interactions that are yet to be discovered. Importantly, Frost et al. (2008) report that membrane tubulation can be a probabilistic outcome, not a predetermined result: K66E mutation produces both flat sheets and tubules in comparable proportion. If both outcomes are possible, the tubule patterns can be kinetically biased, for example during the process of crystallization. Extensive variability in the F-BAR coats not subjected to annealing (Frost et al., 2008) might indicate reciprocal mobility of the proteins within the coat, a characteristic of fluid-like arrangements (Shnyrova et al., 2007). Such a possibility of loose internal ordering within a protein coat is also suggested by a combination of vesiculation and tubulation activity of N-BAR (Gallop et al., 2006). Clearly, studies of membrane remodeling by BAR domains promise many more fascinating chapters in this remarkable story.

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Wnt Signaling in the Niche

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There is much interest in understanding the signals in the bone marrow niche that keep hematopoietic stem cells (HSCs) in a quiescent state. In the current issue of *Cell Stem Cell*, Fleming et al. (2008) report that blocking Wnt signaling in the niche increases the number of proliferating HSCs and reduces their ability to reconstitute the hematopoietic system of irradiated recipient mice. These findings show that Wnt/β-catenin activity is crucial for the maintenance of HSC quiescence in the bone marrow niche.

The interaction of stem cells with their specialized microenvironment or "niche" is critical for their maintenance over long periods. Indeed, it is well established that the niche regulates stem cell-specific properties including self-renewal, multipotentiality, and quiescence. In the hematopoietic system maintaining hematopoiesis throughout life requires balancing the self-renewal and differentiation of pluripotent hematopoietic stem cells (HSCs) in the bone marrow. According to recent studies, the niche for long-term repopulating HSCs in the bone marrow consists of two parts: the endosteal surface (the osteoblast niche) and a sinusoidal endothelium (the vascular niche). New work by Fleming et al. (2008) in mice suggests that Wnt signaling in the bone marrow niche helps to maintain the HSC pool throughout life.

Recently, the key signaling molecule Wnt has been implicated in the self-renewal capabilities of HSCs. The Wnt protein binds to a receptor complex consisting of a member of the Frizzled family of seven transmembrane proteins and the LDL receptor-related proteins LRP5 or LRP6 (Clevers, 2006). In the canonical Wnt pathway, receptor activation leads to stabilization of β-catenin, which accumulates and translocates to the nucleus where it activates target gene expression in concert with transcription factors such as Tcf and Lef.

Fleming et al. (2008) now further analyze the role of Wnt signaling on HSC activity, including its effects on cell-cycle quiescence and the capacity of HSCs to reconstitute the hematpoietic system of recipient mice (whose bone marrow has been ablated by radiation). In contrast to previous studies that genetically manipulated the HSCs themselves, Fleming et al. analyzed the effects of blocking Wnt signaling in the mouse bone marrow microenvironment by overexpression of dickkopf1 (Dkk1), an antagonist of Wnt/ β-catenin signaling. Dkk1 is a soluble secreted protein that interacts with the Wnt coreceptors LRP5 and LRP6 (Kawano and Kypta, 2003). It is known that the number of osteoblasts directly affects the number of long-term repopulating HSCs (Calvi et al., 2003; Zhang et al., 2003). In the new study, transgenic mice expressed Dkk1 in the osteoblastic lineage under the control of a 2.3 kb fragment of the collagen 1α promoter. The overexpression of Dkk1 reduced activation of the Tcf/Lef transcription factors in HSCs in a non-cell-autonomous manner. The transgenic mice showed no significant alteration in the proportion of HSCs and common lymphoid progenitor cells under steady-state conditions. Although HSCs from the Dkk1 transgenic mice could reconstitute the hematopoietic system of irradiated recipient mice, they lost their reconstituting capacity after repeated bone marrow transplantation, indicating that the inhibition of Wnt signaling in the niche results in the premature loss of self-renewal activity. Interestingly, wild-type HSCs also showed a drastic reduction in hematopoietic reconstitution activity of the hematopoietic system in Dkk1 transgenic recipient mice. HSCs undergo extensive proliferation after bone marrow transplantation; Wnt/ β-catenin signaling may play a